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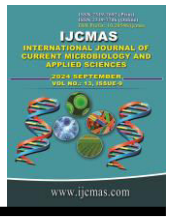

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Parental Polymorphism Survey Between Heat Tolerant N22 and Heat Susceptible 166-30S Using SSRs in Rice

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A B S T R A C T

Molecular marker technology stands out as a pivotal innovation to accelerate the progress in modern rice breeding, contributing to the development of improved varieties that can meet the challenges of global food production and climate change. Markers such as SSRs provide the broad genetic resolution needed to locate QTLs fairly accurately, which can then be easily used in breeding programs to improve crop traits. Mapping QTLs for traits associated with heat tolerance is important to develop heat resilient rice varieties. In the present study polymorphic markers were identified between two highly contrasting heat tolerant rice lines the aus variety N22 and heat susceptible Swarna x *O. nivara* introgression line 166-30S. A total of 748 randomly selected SSR markers covering all the 12 chromosomes were assessed and 110 distinctly polymorphic markers identified.

Introduction

Rice (*Oryza sativa*) is a globally significant crop serving as a staple food for half of the world's population, especially in east and south east Asia. The group of rice varieties which are aus-type exhibit unique stress tolerance traits, making them valuable for rice breeding. Traditional aus- type rice varieties are proven as highly tolerant to environmental stresses like heat and drought, thus serving as an important genetic resource for crop improvement (Sar *et al.,* 2024).

Heat stress poses significant threat to rice cultivation, especially as climate change intensifies. High temperatures result in harsh effects on rice production, in particular during critical growth stages such as booting, heading and grain filling (Zhang *et al.,* 2018). When high temperatures coincide with these critical stages it may lead to sterile pollen, reduced pollen shedding and poor pollen germination and reduced seed set (Jagadish *et al.,* 2010, 2014). Thus understanding the genetic basis of heat tolerance and developing heat tolerant lines/varieties is important to address vulnerability of rice to high temperature. Heat stress tolerance in rice is a crucial trait, especially in face of climate change. While N22 is an aus-type variety, well-known for its heat tolerance at different stages (Vishnu Kiran *et al.,* 2012; Sailaja *et al.,* 2014; Sailaja *et al.,* 2015; Prasad *et al.,* 2006; Jagadish *et al.,* 2011 and Poli *et al.,* 2013). In contrast, 166-30S is an inbreed line with good agronomical characters along with

heat susceptible nature selected as a recurrent parent to identify heat tolerance QTLs in a mapping programme (Prasath *et al.,* 2016). 166-30S is a high yielding line with low spikelet sterility and it was crossed with donor N22 to develop a mapping population for further assessment.

Breeders and researchers are developing varieties that are heat tolerant using associated genetic markers. Many researchers are exploring keys to heat tolerance by mapping quantitative trait loci associated with heat tolerance using various molecular markers, including Simple sequence repeats (SSR). SSR markers, also known as simple sequence repeats or microsatellites are specific DNA sequences found in the genomes of various organisms. SSRs are used as molecular markers for individual identification, parentage analysis and genetic mapping. They help study genetic diversity, relatedness and population structure. They can be used to distinguish between closely related species. Highly polymorphic showing variations in repeat lengths among individuals with co dominant nature (Singh *et al.,* 2024). Breeders can use SSR markers to track multiple genes associated with heat tolerance simultaneously and thus create rice varieties with enhanced heat resilience by combining favourable alleles from different sources. Parental polymorphism detection is a critical first stepto assess genetic variation between donor and recipient parents for the purpose of mapping and subsequent use in marker assisted selection. Polymorphic markers help to track important traits in introgression programs and help in early identification of desired plants.

The purpose of this study was to identify polymorphic markers between two parents N22 and 166-30; examine their chromosomal distribution using physical distance and look for repetitive motifs. With the aid of this study, these identified polymorphic markers can be used for further QTL mapping strategies to identify heat tolerance associated traits.

Materials and Methods

Plant material used in this study

The experimental plant material for this current study consists of two highly contrasting rice parents for desirable heat tolerance traits. N22 a well know Aus type genotype was used a donor parent for its proven heat tolerance based on previous studies.166-30s an inbred line developed from cross between Swarna and *O.nivara* was used as a recurrent parent for its high yielding and good agronomical characters besides its heat susceptible nature.

Genotyping

The genomic DNA was isolated from young leaves of the donor and recurrent rice genotypes using CTAB method (Doyle and Doyle, 1987). The quality and quantity of extracted DNA was estimated using 0.8% agarose gel electrophoresis (Alpha imager UV gel documentation system) and Nanodrop (ND100 spectrophotometer, nanodrop technologies inc., USA). DNA samples were then diluted according to the experiment requirement based on concentration. The parental polymorphism studies were conducted between two contrasting parents using 748 SSR markers. The amplified allies were scored for polymorphism and the resulted polymorphic primers data like chromosome number and physical location was collected from www.gramene.org.

The polymerase chain reaction (PCR) was carried out in thermal cycler (Applied Bio systems, USA) using 1125 SSR markers. The PCR reaction mix consist of 20-50ng of genomic DNA, 1x Buffer (containing 1.5 mM MgCl2), 200 µM of dNTPs, 10 pmolof each (forward and reverse) primer and 0.5 unit of Taq DNA polymerase (Bangalore Genei, India).The PCR profile was included with initial denaturation at 94˚C for 5 min followed by 35 cycles of denaturation at 94˚C for 30 s, annealing at 55˚C for 30 s and extension at 72˚C for 1 min and final extension of 10 min at 72˚C. The PCR product was resolved in a 3% agarose gel stained with ethidium bromide prepared in 1X TAE buffer. The electrophoresed products were visualized under UV light and documented using Alpha lmager Documentation System (M/s Alpha Innotech, USA).

Software analysis

The parental polymorphism between the two parents; N22 and 166-30 was recorded based on the different base pair size of both parents. Polymorphic % was calculated by using the following formula:

$$\text{Polymorphism \%} = \frac{\text{Number of polymorphic markers identified per chromosome}}{\text{Total number of markers used per chromosome}} \times 100$$

GGT software was used to conduct the linkage map of resulted polymorphic markers using their respective chromosome number and physical location data retrieved.

Results and Discussion

The occurrence of two or more discontinuous alleles of gene on a specific locus in a given population is referred as genetic polymorphism. The analysis of polymorphic primers associated with heat tolerance in crossing parents and cross progeny needs to be conducted. The rate of gene polymorphism is estimated to be less than or equal to 1% (Howell *et al.,* 2002). Parental polymorphism at the molecular level was determined by genotyping the two parents with SSR markers. The genomic DNA of the two parents N22 and 166.30 were initially screened with 748 Rice microsatellite markers (RM). Out of 748, only 110 markers showed clear polymorphism. Among these 17 markers were on chromosome 2 as highest and 6 markers each on chromosome 6 and seven as lowest polymorphism. The total list of polymorphic markers along with their respective chromosomes is illustrated as graph (Fig.1). The linkage map was constructed using physical positions of polymorphic markers (Fig.2). The percent of polymorphism between any parents depended on the number of relevant primers used for study. The present parental survey revealed 14.7% of polymorphism. Similar to the findings of this study, Wei *et al.,* (2013) had identified 13.98 percent polymorphism between HT54 (heat tolerant) and HT13 (heat susceptible) parents. Waghmare *et al.,* (2018) observed 20.82 % polymorphism among two parents N22 and Uma. Zhang *et al.,* (2009) had identified 30 % polymorphism between heat tolerant 996 and heat susceptible 4628. Buu *et al.,* (2014) observed 52.6% polymorphism between heat tolerant N22 and heat susceptible OM5930. The identified 110 rice microsatellite markers between parents will be useful as a pointer to the existence of allelic difference at each locus. As the two parents differ from each other with respect to various agronomical traits along with heat stress, the identified polymorphic markers will be useful in identification and mapping heat tolerance QTL mapping. Out of these 110 polymorphic markers, 12 markers were recognised by earlier researchers as associated with QTLs for heat tolerance in rice.RM224 was a polymorphic marker between parents for heat tolerance as identified by Nguyen *et al.,* (2022). Likewise, Stephen *et al.,* (2023) reported RM337 and RM 470 as polymorphic markers, Buu *et al.,* (2014) was identified RM174, RM228, RM209, Bharathkumar *et al.,* (2014) reported RM341, Zhao *et al.,* (2006) reported RM 153, Casartelli *et al.,* (2018) reported RM440, Zhao *et al.,* (2006) reported RM447, Ye *et al.,* (2012) reported RM547, Cao *et al.,* (2003) reported RM 348, Prasanth *et al.,* (2016) reported RM 250 and Wei *et al.,* (2013) reported RM219 to be associated with heat tolerance.

Figure.1 List of markers polymorphic between N22 and 166-30S

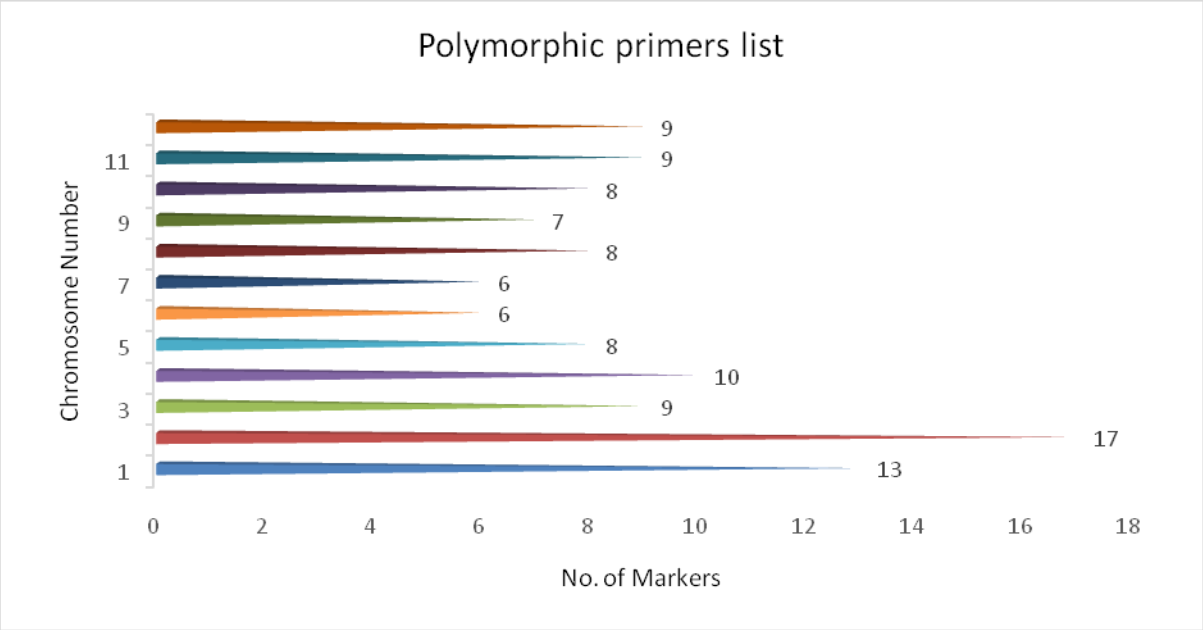
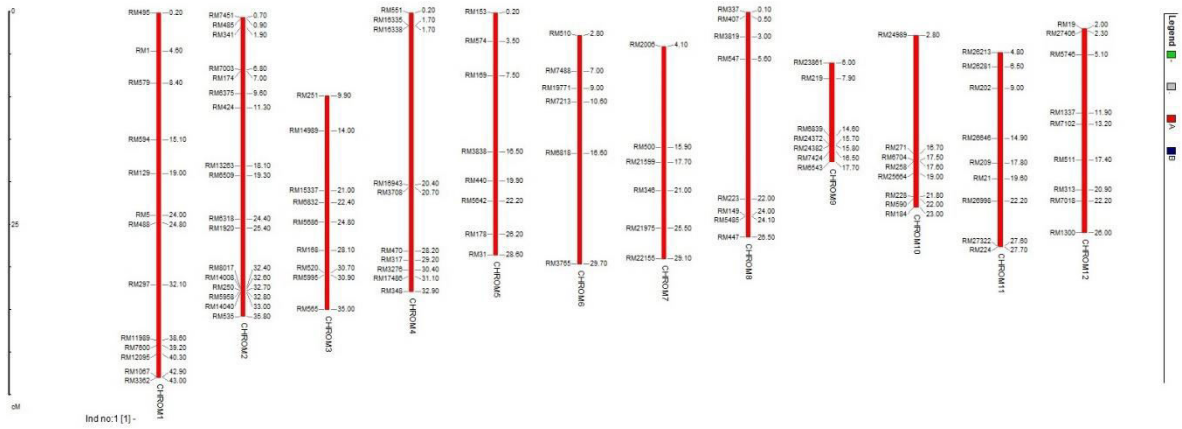


Figure.2 Linkage map of polymorphic markers identified using physical location (Mb)



The resulted polymorphic markers can be utilised to perform QTL analysis of various heat stress related traits along with developed mapping population. 166-30; besides its heat susceptible nature is agronomically better performance than Swarna. N22 is a well-known heat tolerant donor parent can be utilized to study heat associated traits and QTLs with the RIL mapping population using the susceptible 166-30. By using these polymorph/v/cicmarkers, we can map QTLs associated with heat tolerance. More over these identified polymorphic markers can be utilized in various studies like marker assisted breeding, diversity analysis and linkage analysis for various traits in rice.

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Author Contributions

M. Suchandranath Babu: Investigation, formal analysis, writing—original draft. V. Vishnu Prasanth: Validation, methodology, writing—reviewing. T. Vishnu Kiran:—Formal analysis, writing—review and editing. Satendra K. Mangrauthia: Investigation, writing—reviewing. S. R. Voleti: Resources, investigation writing—reviewing. P. Sudhakar: Validation, formal analysis, writing—reviewing. A. Krishna Satya: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript. Sarla Neelamraju: Investigation, formal analysis, writing—original draft

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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